DATA EVALUATION RECORD

STUDY 1

CHEM 112600 Prohexadione calcium §161-1
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Singh, M. 1995. Hydrolysis of prohexadione calcium in aqueous media. BASF Report No.: M9502. BASF Reg. Document #95/5186. Unpublished study performed and submitted by BASF Corporation, Research Triangle Park, NC.

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REVIEWED BY:	D. R. Hunt, B.A.	Signature:
TITLE:	Scientist	Date:
EDITED BY:	C. A. Little, Ph.D.	Signature:
TITLE:	Sr. Scientist/Asst. Project Manager	Date:
APPROVED BY:	P. H. Howard, Ph.D.	Signature:
TITLE:	Project Manager	Date:
ORG:	Syracuse Research Corp. Arlington, VA 22202	
TEL:	703/413-9369	

APPROVED BY:	Iwona L. Maher, Chemist	James A. Hetrick, Ph.D., Chemist
ORG:	ERB I/EFED/OPP	ERB I/EFED/OPP
TEL:	703/605-0569	703/305-5237
DATE:	7/26/2006	
SIGNATURE:	Iwona Maher	James A. Hetrick

CONCLUSIONS

Degradation - Hydrolysis

1. This study is scientifically valid and satisfies the Subdivision N data requirement for a hydrolysis study.

2. [3,5-^{14}C]prohexadione calcium hydrolyzed with respective half-lives of 4.4 days (r^2 = 0.99) and 65.0 days (r^2 = 0.98) in pH 5 and pH 7 sterile aqueous buffer solutions incubated in darkness at 25 ± 1 °C for up to 671 hours. In sterile pH 9 aqueous buffer solution [3,5-^{14}C]prohexadione calcium was hydrolytically stable.

3. The major hydrolysis degradate, 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid, was present at a maximum concentration of 91.6% at pH 5 and 24.1% at pH 7, at 671 hours posttreatment.

METHODOLOGY

Cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium {BAS 125W; calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate; radiochemical purity 98.6%, specific activity 15.3 mCi/mmol; p. 8}, dissolved in acetonitrile:water (1:1, v:v), was added at concentrations of 6.33 ppm (pH 5), 6.32 ppm (pH 7), and 6.2 ppm (pH 9) to bulk pH 5 (citrate), pH 7 (phosphate), and pH 9 (borate) sterile aqueous buffer solutions (pp. 9, 10). Buffer solutions were autoclaved prior to treatment, and sterility was confirmed on agar plates. The treated buffer solutions were transferred to brown glass ampules and the ampules were sealed and placed in a water bath maintained at 25 ± 1 °C in darkness for up to 671 hours (28 days). Individual samples were removed for analysis at 0, 24, 30 (pH 5 only), 48, 54 (pH 5 only), 72, 94, 96 (pH 5 only), 166, 214, 262, 358, 406, 503, 575, and 671 hours posttreatment.

Duplicate aliquots of the bulk treated buffer solution remaining following distribution to ampules were analyzed for total radioactivity by LSC to determine time 0 concentrations (p. 10). At each sampling interval, except time 0, duplicate aliquots of the samples were analyzed for total radioactivity by LSC (p. 11; Reviewer's note: the limits of detection and quantitation were not reported). An aliquot of each test solution was analyzed at each sampling interval by HPLC (PRP-1 column) with a mobile phase gradient of water plus 0.25% acetic acid:acetonitrile (95:5 to 10:90 to 5:95, v:v) with UV detection (264 nm; pp. 11, 30). Eluate fractions were collected at 15-second intervals and analyzed by LSC. To confirm compound identities, additional samples of pH 5 buffer solution were prepared and treated with the parent at 3.2 ppm (pp. 11, 12). The samples were incubated for 5 days as described previously, then removed and analyzed by HPLC with fraction collection as described above. The remaining residue was lyophilized and the sample was redissolved with water:3% ammonium hydroxide (19:1, v:v). Fractions containing [^{14}C]residues as determined by LSC were concentrated and analyzed by HPLC as described

above with co-chromatography of nonradiolabeled reference standards of the parent and the suspected degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid (R3). Additional compound identification for the parent and major degradate (R3) was performed on methylated samples of fractions containing [^{14}C]residues using GC/MS in the electron impact mode (p. 15).

The temperature of the water bath was measured at each sampling interval and the pH was measured in samples collected at 0, 262, and 671 hours posttreatment. The temperature ranged from 24.6 to 25.5 °C from 0 to 671 hours posttreatment and the pH was 5.0, 7.0, and 9.0 for each of the respective buffer solutions at each sampling interval (Appendix 1; Tables 1, 2; pp. 42, 43).

STUDY AUTHOR'S DATA SUMMARY

Cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium (BAS 125W; radiochemical purity 98.6%), at a concentration of 6.3 ppm, hydrolyzed with respective registrant-calculated half-lives (derived from equation $t_{1/2} = \ln 2/k$, where k was determined from a least squares linear regression method of $\ln (c_o/c)$ versus time plot) of 4.4 days ($r^2 = 1.0$) and 65.0 days ($r^2 = 0.99$) in pH 5 and pH 7 sterile aqueous buffer solutions incubated in darkness at 25 ± 1 °C for up to 671 hours (Figures 4, 5; pp. 31, 32; Table 8, p. 27). The parent, at 6.2 ppm, was hydrolytically stable in sterile pH 9 aqueous buffer solution (Figure 6, p. 33).

In the pH 5 buffer solution, the parent (R5 in data tables), which was initially present at 95.8% of the applied radioactivity, decreased to 49.1% by 94 hours and 20.7% by 214 hours, and was 1.4% at 671 hours posttreatment (Table 2, p. 21). The major degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid (despropionyl, BW9054-5376; R3 in data tables) was initially (time 0) present at 3.4% of the applied radioactivity, increased to 46.1% by 94 hours and was a maximum of 91.6% at 671 hours posttreatment. Two unidentified minor degradates were present at maximums of 6.4% (R4 at 262 hours) and 1.1% (R2 at 214 hours) of the applied radioactivity.

In the pH 7 aqueous buffer solution, the parent (R5) was initially present at 97.7% of the applied radioactivity. It was present at 84.2% at 262 hours and decreased to 72.9% by 671 hours posttreatment (Table 3, p. 22). The major degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid (R3) was initially (time 0) present at 1.4% of the applied radioactivity. It increased to 10.9% by 262 hours, and was a maximum of 24.1% of the applied at 671 hours posttreatment. Two unidentified minor degradates were present at maximums of 2.5% (671 hours) and 0.85% (406 hours) of the applied radioactivity.

In the pH 9 aqueous buffer solution, the parent (R5) was present at 95.8% of the applied radioactivity at 671 hours posttreatment (Table 4, p. 23). The minor degradate, 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid (R3), was present at 1.4 to 1.9% of the applied radioactivity from 0 to 671 hours posttreatment. Two unidentified minor degradates were present at maximums of 3.7% (575 hours) and 1.0% (94 hours) of the applied radioactivity.

Material balances, based on HPLC analysis, were 97.5-100.0% (pH 5), 96.0-100.0% (pH 7), and 100.0-103.9% (pH 9) of the applied radioactivity, throughout the incubation period (Tables 2-4, pp. 21-23).

THE REVIEWERS' COMMENTS

1. The hydrolysis study data indicated that the parent compound is less hydrolytically stable as the acidity decreases from pH 9 to pH 5. ^{14}C-Prohexadione calcium, (BAS 125W) at a concentration of 6.3 ppm incubated in darkness at 25 ± 1 °C, hydrolyzed with the half-lives of 4.4 days (derived from linear regression, $r^2 = 0.99$) and 65.0 days ($r^2 = 0.98$) in pH 5 and pH 7 (sterile aqueous buffer solutions), respectively. It was hydrolytically stable in sterile pH 9 aqueous buffer solution.

2. The half-life (65.0 days) of the parent in the pH 7 system was estimated assuming the continuation of the apparent degradation pattern beyond the scope of the observed data. However, data which appeared linear may become curvilinear with time and half-life estimations based on extrapolated data may be inaccurate.

3. A phosphate buffer was utilized to study the hydrolysis of the test compound at pH 7 (pp. 9, 10); it is recommended that borate or acetate buffers be utilized to minimize catalysis effects of the buffer. In addition, the molarity of the buffer solutions was not reported, as required by Subdivision N Guidelines. The reviewer noted that the buffer solutions were prepared from premixed concentrates that were diluted (pp. 9, 10).

4. The study authors prepared the prohexadione calcium dosing solution in acetonitrile:water even though the solubility much higher than 10 ppm. The aqueous solubility of the parent compound at 20 °C was 174.0 ppm in distilled water (DW), 1602 ppm in pH 5 buffer solution, 786 ppm in pH 7 buffer solution, and 665 ppm pH 9 buffer solution (p. 8). Therefore a solubilizing agent is not necessary to increase the chemical water solubility.

5. Method detection limits (MDL) and limits of quantitation (LOQ) were not reported for the prohexadione calcium and its degradates.

Prohexadione calcium

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